REMARKS

Objection to the Declaration

The Applicants note the Examiner's comments with regard to the allegedly defective Declaration. A substitute Declaration is currently being prepared and will be submitted for consideration in due course.

Objections to the Specification

The objections to the Specification are obviated in part by appropriate amendment, and traversed in part.

The Examiner has requested an explanation of certain changes made to the Specification by way of the Preliminary Amendment filed on January 22, 2002.

Following entry of the present Amendment to the Specification, the paragraph at page 5, lines 19-21, now reads:

"According to another feature, the invention relates to dendritic cells that are $\alpha v\beta_3^-$, $\alpha v\beta_5^+$, $CCR5^-$ and $CCR7^+$, i.e. are devoid of $\alpha v\beta_3$ and CCR5 receptors and carry $\alpha v\beta_5$ and CCR7 receptors."

In essence, the term "i.e. are devoid of $\alpha v\beta_3^-$" that appeared in the application as filed, has been changed to read "i.e. are devoid of $\alpha v\beta_3$". Quite clearly, the "i.e." language is merely stating the obvious: that dendritic cells that are phenotypically "$\alpha v\beta_3^-$, $\alpha v\beta_5^+$, $CCR5^-$ and $CCR7^+$", are in fact devoid of $\alpha v\beta_3$ and CCR5 receptors, whilst they carry $\alpha v\beta_5^+$ and CCR7 receptors.

With regard to the change of "4 μm/ml of CHX" to "4 μg/ml of CHX" on page 12, line 24, of the Specification, Applicants respectfully submit that an error occurred when the French language International Application No. PCT/FR00/02173 was translated into English for filing in the U.S. As can be seen from page 14, line 5, of International Publication No. WO 01/09288 (attached), the original text as filed recited "4 μg/ml de CHX", which obviously translates to 4 μg/ml of CHX".

With regard to the Examiner's remarks in connection with the marked-up version of the Specification at page 7, lines 22-27, the correct changes are shown by way of the present amendment.

Rejections under 35 U.S.C. 112, second paragraph

The rejection of claims 14-22 under 35 U.S.C. 112, second paragraph, is obviated in part by appropriate amendment, and traversed in part.

A) Applicants submit that the term "mobilization" is clear and concise to a person of ordinary skill in the art, and refers to the release of cells into the circulatory system following physical, environmental, or other, factors impacting on the immune system.

B) Claim 17 has been cancelled.

C) Claim 20 has been amended to recite "an interleukin that blocks differentiation towards the macrophagic pathway", which has clear antecedent basis in claim 14 from which claim 20 depends.

In view of the above amendments and remarks, Applicants submit that the present claims are clear and concise to a person of ordinary skill in the art. Accordingly, withdrawal of the rejection under 35 U.S.C. 112, second paragraph, is urged.

Rejections under 35 U.S.C. 112, first paragraph

The rejection of claims 14-17 and 19-22 under 35 U.S.C. 112, first paragraph, is respectfully traversed.

Firstly, the Examiner has objected to the term "an interleukin that blocks differentiation towards the macrophagic pathway" in claim 14. The Examiner has acknowledged that the specification recites IL-4 and IL-13 as being representative of such interleukins, but has articulated that the specification provides no further guidance to enable the skilled artisan to identify other interleukins for use in the claimed invention.

Similarly, the Examiner has articulated that the specification provides insufficient enablement for the term "cell growth factor" in claim 19.

Applicants respectfully submit that the specification is enabling for the terms "an interleukin that blocks differentiation towards the macrophagic pathway" and "cell growth factor".

The Applicants have disclosed that interleukin-4 and interleukin-13 are examples of interleukins that can be used in the invention (page 3, lines 14-20).

The Applicants have also provided examples of cell growth factors, namely granulocyte colony stimulating factor (G-CSF) and granulocyte-macrophage colony stimulating factor (GM-CSF) on page 4, lines 28-33, of the specification.

It is respectfully submitted that such information would provide guidance to those of ordinary skill in the art to enable them to practice the claimed invention without undue experimentation.

Moreover, the burden is on the Patent Office to provide reasons and/or examples in support of their belief that the presumptively enabling disclosure does not support the claimed invention. Since the Examiner has provided nothing but mere conjecture, while Applicants have provided clear examples of the interleukins and cell growth factors that can be used in the invention, this invention is clearly enabled to those of ordinary skill in the art. Accordingly, withdrawal of the rejection under 35 U.S.C. 112, first paragraph, is urged.

Rejections under 35 U.S.C. 103(a)

The present invention relates to a method for obtaining dendritic cells, which comprises:

1) cultivating for 4 to 6 days, preferably 5 days, mononuclear cells derived from cytapheresis after mobilization, in a serum-free medium supplemented with human albumin at a rate of 1 to 2 % w/v of medium, in the presence of a granulocyte-macrophage colony stimulating factory (GM-CSF) and an interleukin (IL) that blocks differentiation towards the macrophagic pathway;

2) adding TNF-α and optionally an inflammatory mediator to the culture medium and continuing the culture for about a further 1 to 4 days, preferably 2 days ; and

3) recovering the dendritic cells formed.

This method allow the obtention in a reproducible manner of cells having:

– a high viability;

– a membrane phenotype characteristic of dendritic cells (absence of CD 14, presence of CD 83, important density of MHC classes I and II, presence of co-stimulating molecules CD 80 and CD 86), and

– biological properties which are characteristic of dendritic cells (endocytosis of exogenous particles, phagocytosis of apoptotic cells, production of IL-12 type cytokines, expression of chemokine receptors and response to chemokines present in the ganglions, stimulation of allogenic T lymphocytes).

The rejection of claims 14-17 and 19-22 under 35 U.S.C §103 (a) as being unpatentable over the Tarte et al. reference in *Blood*, 91: 1852-1857 (1980) in view of Ponting (US 5,405,772) is respectfully traversed.

None of these relied upon references disclose or suggest the invention as claimed.

Tarte et al. discloses a method for obtaining dendritic cells in a X-VIVO 15 medium alone. The cells obtained are of poor viability and are incompatible with an immunotherapeutical treatment.

In contrast, in the method of the presently claimed invention the medium is supplemented with human albumin at a rate of 1 to 2 % w/v of medium. The results of Example 1 of the present invention show that the addition of human albumin considerably increases the viability of the cells produced in X-VIVO 15 medium alone.

Table A shown in the attachment gives the viability percentages of the dendritic cells obtained starting from adhering cells of peripheral blood cultured during 5 days in presence of

GM-CSF and IL-4 in RPMI 1640 medium supplemented with 10 % of foetal calf serum (FCS), in X-VIVO 15 medium alone or in X-VIVO 15 supplemented with 2 % of human albumin.

The above results show that the use of 2 % of human albumin in the X-VIVO 15 medium allows the obtention of viable dendritic cells compatible with an immuno-therapeutic treatment. The human albumin is needed for obtaining dendritic cells on a serum-free medium.

The dendritic cells have the biological characteristics for activating the immune response in a ganglion as shown by the results of Table 1 of the present application and by the results of Table B (attached) hereinafter concerning the properties of dendritic cells produced by cultivating adhering cells of peripheral blood of 33 patients in a X-VIVO 15 medium complemented with human albumin, in presence of GM-CSF and IL-4.

Accordingly, it was not obvious on the basis of the teachings of Tarte et al and Ponting that dendritic cells with a high degree of viability could be obtained under the culture conditions as defined in claim 1 with the specific amount of human albumin of 1 to 2 % (w/v).

For the reasons stated above, the Tarte et al. and Ponting references do not teach or suggest the invention defined by the present claims. Accordingly, the rejection under 35 U.S.C. 103(a) should be withdrawn.

The rejection of claim 18 under 35 U.S.C §103 (a) as being unpatentable over Tarte et al. in view of Ponting and further in view of Kalinski et al. is respectfully traversed.

For the reasons expounded above, the Tarte et al. and Ponting references do not teach or remotely suggest the invention defined by the present claims.

The Kalinski et al. reference does not fill the gaps left by Tarte et al. and Ponting.

Kalinski et al. points out the role of PGE_2 in the maturation of dendritic cells in a medium with 10 % of foetal calf albumin (see materials and method). Applicants submit that it would not have been obvious to a person of ordinary skill in the art that in a serum-free medium, the PGE_2 would have the indicated action, as explained on pages 6 and 7 of the Specification.

For the reasons stated above, the Tarte et al., Ponting, and Kalinski et al. references do not teach or suggest the invention defined by claim 18. Accordingly, the rejection of claim 18 under 35 U.S.C. 103(a) should be withdrawn.

In view of the deficiencies in the art, claims 14-22 are not *prima facie* obvious to one of ordinary skill in the art and, accordingly, withdrawal of the rejections under 35 U.S.C. 103(a) with respect to these claims is respectfully requested.

Applicant submits that the present application is now in condition for allowance and early notice of such action is earnestly solicited. If any final points remain that can be clarified by telephone, Examiner G. R. Ewoldt is respectfully encouraged to contact Applicant's attorney at the number indicated below.

Applicants hereby petition the Commissioner for Patents to extend the time for reply to the Office action mailed on April 25, 2005, for three (3) months from July 25, 2005, to October 25, 2005. A duly completed credit card authorization form is attached to effect payment of the extension fee.

Respectfully submitted,

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Table A

	RPMI1640- 10%FCS	X-VIVO15	X-VIVO15+2% human albumin
Viability of cells (%)	90 97 99 92 90 87 98 92 92 97.7	67 70 93 50 65 50	89 81 86 94 93 93.1 90.1 99.3 93.2 92
Average ±	93.5 4.1	65.8 15.9	91.1 5.0
		---------- P < 0.05 ----------	

Table B

	Viability	CD14	CD80	CD86	HLA class I	HLA class II
	96.7	56.4	91.1	76.7	99.2	92.7
	90.1	3.1	85.9	72.6	96.4	96.5
	90	4.5	93.4	49	93.5	95.3
	95	13	94.6	78.2	99.8	98.8
	97	1.8	72.1	58.2	99.8	92
	99	20.8	87.1	69.6	99.8	96.1
	95.4	13.2	94.8	79.5	99.6	94.9
	93.1	2.1	97.8	84.5	99.9	99.6
	93.2	2.5	86.9	92.5	98.5	98.5
	94	0	88.2	91.4	99.4	83.8
	95.8	18.7	92.3	86.2	99.9	92.7
	96.6	2	86	30	99	98
	97.8	7.7	83.9	79.5	99.8	99.1
	99.3	23.9	96.8	72.6	99.9	98
	94.5	12.6	91.8	79.9	99.9	97.9
	96.5	3.4	81.7	35.8	*	*
	95.9	25	94.5	95.4	99.6	99.5
	98	2.4	97.8	51.2	99.9	93.8
	94.8	0.2	96.6	72.3	99.9	96.1
	93.2	0.4	87.3	66.3	99.3	95.6
	93.6	1.3	93.5	77.4	99.5	96.8
	97	2.9	91.5	49.9	96.8	99.8
	93	0	91.5	49.3	98.8	95.8
	92	0	89.3	96.9	94.1	96.2
	96.3	0	90.9	97.6	97.4	90.3
	97.2	3.5	87.3	91.3	*	92
	92.3	1	87.5	84.7	*	95.1
	96.4	0.5	55.9	56.2	*	83.1
	96.3	1.3	72.3	52.1	*	80.7
	96.3	11.9	80.5	69.7	*	81.2
	94.1	6.5	91.5	60.5	*	89.9
	94.2	0	94.8	80.6	98.9	98.6
	93.7	5.6	80.7	89.8	98.9	90.6
	89.2	0	75	85.9	98.1	97.5
Average	94.9	7.3	87.7	72.5	98.7	94.1
±	2.5	11.3	8.8	17.8	1.7	5.3

cellules tumorales à trois reprises dans du X-VIVO 15-2 % de HA avant de les mettre en coculture avec des DC obtenues selon le mode opératoire décrit à l'exemple 1.

Les résultats obtenus sont reportés sur la figure 2. Ces résultats montrent qu'après 6 h de culture avec 4 µg/ml de CHX, 60 % des cellules de myélome XG-1 ont montré des caractéristiques de mort cellulaire apoptotique précoce, c'est-à-dire une liaison de Annexin-V mais une non-incorporation de PI.

EXEMPLE 5 : Phagocytose des cellules apoptotiques

La phagocytose des cellules apoptiques constitue un autre mode d'entrée des antigènes et joue un rôle majeur dans le phénomène d'amorçage croisé. Récemment, plusieurs récepteurs phagocytaires ont été identifiés sur les DC obtenues en présence des sérums humains et il a été montré qu'un milieu conditionné pour des monocytes (MCM), qui conduit à une maturation des DC irréversible, régule en aval leur expression (6).

On a teinté en vert les DC immatures et matures en utilisant du PKH67-GL (Sigma) et on les a cultivées pendant 2 h pour permettre la libération du colorant non lié. On a teinté en rouge des cellules XG-1 en utilisant du PKH26-GL (Sigma) selon les instructions du fabricant avant leur induction pour subir l'apoptose par CHX pendant 6 à 8 h. Ensuite, on a cocultivé les cellules XG-1 teintées en rouge avec des DC immatures ou matures teintées en vert dans un rapport de 1:1 dans du X-VIVO 15-2 % de HA selon le protocole décrit par Albert et al. (6) . Après 90 min à 37°C, on a analysé les fluorescences vertes et rouges avec un appareil FACScan. Dans les expériences de blocage, on a co-incubé les cellules XG-1 et les DC à 4°C.

Les marqueurs CD36, $\alpha v\beta 3$ et $\alpha v\beta 5$ ont été déterminés selon la méthode par marquage par anticorps monoclonaux et cytométrie de flux.

Pour la coloration de $\alpha v\beta 5$, on a tout d'abord incubé les cellules avec un anticorps mAb primaire $\alpha v\beta 5$ (Chemicon Int, Temecula, CA), puis avec un anticorps de chèvre anti-Ig de souris conjugué à FITC (Immunotech). On a réalisé les analyses avec un appareil FACScan (Becton Dickinson).

Les données provenant d'une expérience représentative parmi 3 sont représentées sur la figure 3. Plus d'un tiers des DC immatures ont englouti des XG-1 apoptotiques après 90 min de coculture. Seuls 10 à 12 % des DC immatures ont été teintées deux fois après coculture avec des cellules XG-1 non-apoptotiques. La phacocytose des cellules tumorales par des DC immatures